Ecotoxicology and Environmental Safety 164 (2018) 344–354

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Differential response of quinoa genotypes to drought and foliage-applied H_2O_2 in relation to oxidative damage, osmotic adjustment and antioxidant capacity

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ARTICLE INFO

Keywords:
Antioxidant enzymes
Drought stress
Osmolytes
Osmotic adjustment
Inorganic ions and K^+/Na^+ ratio

ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.), a highly nutritious grain crop, is resistant to abiotic stresses (drought, salinity, and cold) and offers an alternate crop to endure harsh environmental conditions under the face of climate change. Naturally, quinoa genome displays a wide degree of variabilities in drought tolerance strategies. Therefore, the present study was designed to investigate drought tolerance variations and stress tolerance enhancement in four quinoa genotypes (Pichaman, Colorado-407D, IESP and 2-Want) thorough foliage-applied H_2O_2 with the purpose of identifying suitable genotype for water limited environments. The plants were exposed to two watering regimes (75% and 30% pot WHC) and foliage-applied H_2O_2 treatments (15 mM). The drought stress significantly reduced plant growth, relative water contents, chlorophyll and carotenoids contents and increased ROS production (H_2O_2 and $O_2^{\bullet -}$) resulting in higher oxidative damage in all quinoa genotypes. Besides, drought stress significantly enhanced the antioxidants (SOD, PPO, and PAL) activity, total soluble sugars, proline, AsA contents and increased the total accumulation of measured inorganic ions in all quinoa genotypes. The PCA analysis indicated that parameters related to osmotic adjustment and antioxidant capacity were more pronounced in 2-Want and IESP genotypes, while parameters depicting oxidative damage were higher in Colorado-407D and more specifically in Pichaman. However, foliage-applied H_2O_2 effectively improved the osmolytes accumulation, antioxidants activity and K^+/Na^+ ratio which increased water relations, reduced lipid peroxidation and ultimately resulted in higher plant growth. Overall, 2-Want and IESP genotypes were found relatively more drought resistant, while exogenous application of H_2O_2 can be opted for more improvement in osmotic adjustment and antioxidant system, which may further enhance drought tolerance, even in sensitive genotypes of quinoa, such as Pichaman.

1. Introduction

Water is an essential element which is required for all aspects related to plant growth and productivity. Plant's morphological and physiochemical attributes are adversely affected under water deficit conditions (Jones et al., 2003; Latif et al., 2016). Recently, drought episodes are becoming common and rapid climate change scenario has further increased the risk of drought in arid and semi-arid regions (Farooq et al., 2017), which may threaten the food security. Under this alarming situation, a continuous and even increased food supply must be ensured to feed the future population. In this scenario, a viable option could be to add climate resilient grain crops in our existing food production system. However, an efficient, economical and quicker way is to introduce the underutilized crops such as quinoa, which could be the best choice to meet an increasing food demand even in the challenging environments.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal crop native to Andean region (Waqas et al., 2017; Bazile et al., 2016). Quinoa grain is a highly nutritious food source that comprises of a complete set of essential amino acids like lysine, methionine and threonine, which are

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https://doi.org/10.1016/j.ecoenv.2018.08.004
Received 12 April 2018; Received in revised form 28 July 2018; Accepted 1 August 2018
Available online 18 August 2018
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limited in other common cereals (Filho et al., 2017). Due to its venerated importance for food security, The United Nation has declared and celebrated the year 2013 as "The International Year of Quinoa (IYQ 2013)". Now, quinoa cultivation and experimentation is increasing rapidly throughout the world. At present, more than 95 countries are growing quinoa (Bazile et al., 2016; Peterson and Murphy, 2015). It has the ability to tolerate abiotic stresses such as drought (Amjad et al., 2015; Aziz et al., 2018; Fghire et al., 2013; Iqbal et al., 2018; Razzaghi et al., 2011; Sun et al., 2014), salinity (Hariadi et al., 2010; Shabala et al., 2012; Waqas et al., 2017), frost and even grow well under marginal lands (Bazile et al., 2016), which makes it an emerging stress-tolerant model crop. Normally, the plants have developed an array of several physiological, biochemical and molecular mechanisms to mitigate the deleterious effects of drought stress.

Osmotic adjustment (OA) in terms of osmolytes accumulation has long been considered as an important physiological mechanism in plants for adaptation to drought stress (Blum, 2017). Organic solutes such as soluble sugars, proline, glycine betaine and inorganic ions (K^+, Na^+ and Cl^-) are the key osmolytes which plays vital role in osmoregulation (Shabala and Shabala, 2011; Hessini et al., 2009). Formerly, several works have reported that pronounced accumulation of these osmolytes under water deficit conditions, increase drought tolerance in quinoa (Aziz et al., 2018; Sun et al., 2014; Iqbal et al., 2018; Bascuñán-Godoy et al., 2016), while, accumulation of inorganic ions and their contribution in osmotic adjustment of quinoa under drought stress is still obscured.

Drought stress usually leads to oxidative damage due to increased concentration of reactive oxygen species (ROS) in several plants (Ashraf et al., 2015; Farooq et al., 2017; Iqbal et al., 2018). The ROS such as hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and superoxide ($O_2^{\cdot -}$) are extremely cytotoxic and can seriously disrupt normal metabolism by damaging nucleic acids, oxidizing proteins and causing lipid peroxidation (Foyer and Noctor, 2005, 2009; Alscher et al., 2002). In order to overcome oxidative damage, plants have evolved antioxidative defense systems to create a balance between the production and scavenging of these species (Alscher et al., 2002). The plant's defense system includes enzymatic (SOD, POD, CAT, APX and GPX) and non-enzymatic antioxidants (AsA and GSH) which act together in a sequence to scavenge ROS (Cavalcanti et al., 2007, 2004). It is an eminent fact that antioxidants are the first line of defense against lethal ROS (Amjad et al., 2015), and alleviated level of ROS reduce the oxidative damage and increase drought tolerance in quinoa (Amjad et al., 2015; Aziz et al., 2018; Iqbal et al., 2018).

Previously, contrasting results were found; Fghire et al. (2013) reported that CAT and POD activity were increased, while, Waqas et al. (2017) and Iqbal et al. (2018) reported decreased CAT and POD activity under salt and drought stress in quinoa. This controversy might be attributed to both genetic variation of quinoa genotypes and variances in kinetics of stress development used in these studies.

On the other hand, several studies have reported that exogenous application of stress signaling molecules like hydrogen peroxide (H_2O_2), nitric oxide (NO), hydrogen sulfide (H_2S), melatonin (Mel), and polyamines (Pas) have potent role in increasing stress tolerance by deploying several physiological mechanisms especially improved antioxidants system and pronounced accumulation of osmolytes (Abass and Mohamed, 2011; Ashraf et al., 2015; Farooq et al., 2017; Guler and Pehlivan, 2016; Hossain et al., 2015; Hossain and Fujita, 2013; Savvides et al., 2016).

Among ROS, hydrogen peroxide (H_2O_2) is a small sized, stable molecule which can easily and rapidly diffuse across the subcellular membrane (Bienert et al., 2006). It act as double-edge sword in plant physio-biochemical processes; at higher concentrations it causes programmed cell death and reduces the plant photosynthesis (Dat et al., 2000), while works as secondary messenger and coordinate with many crucial signaling molecules within plant at low concentration (Bienert et al., 2006; Hossain et al., 2015; Shu-Hsien et al., 2005). Foliar application of H_2O_2 at low concentration improved the stress tolerance through increased antioxidant system and accumulation of osmolytes in different crops such as soybean (Ishibashi et al., 2011), marigold (Liao et al., 2012), maize (Terzi et al., 2014) wheat (Farooq et al., 2017) and cucumber (Liu et al., 2010).

Adaptation and tolerance of crop plants to accommodate the drought is generally associated with the induction of antioxidants system, oxidative defense and osmotic adjustment for the protection of several physiological functions. Up to date, underlying genotypic variability of quinoa concerning the antioxidants system, oxidative stress and osmotic adjustment responses under drought conditions is still unknown, and there has been no study reporting the effect of foliage-applied H_2O_2 on drought tolerance mechanisms on different genotypes of quinoa. Therefore, considering the esteemed importance of marvelous grain crop quinoa for food security and keen-sighted role of H_2O_2 in antioxidant system enhancement, the present study aimed (1) to ascertain the variations in antioxidant activity, osmotic adjustment and oxidative damages of four quinoa genotypes in response to drought stress, and (2) to investigate the influence of foliage-applied H_2O_2 in drought tolerance enhancement of quinoa.

2. Materials and methods

2.1. Experimental material

The experiment was conducted at Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China. Four genotypes of Quinoa used in this study were acquired from Alternate Crops lab of the Department of Agronomy, University of Agriculture Faisalabad, Pakistan (Table 1). Seeds were surface sterilized with 1% sodium hypochlorite for 10 min followed by repeated washings with distilled water and then re-dried under shade to obtain original weight. The experiment was conducted in plastic pots, which were filled with 5 kg loam soil on dry weight basis. Soil chemical analysis showed 0.51% organic matter, N-P-K, 70.71, 9.15, and 67.21 mg/Kg, respectively, EC 0.61 dS m^{-1}, and pH 7.65. Five seeds were sown in each pot (10 June) and 10 days after emergence, seedlings were thinned to one plant per pot. All the pots were fertilized with 50% Hoagland solution at 12th and 30th days after sowing. Pots were kept in wire house under natural conditions. Wire house comprised of thin iron rods to support the moveable transparent plastic sheet, used to protect the plants from rain, when needed. The weather conditions prevailed during quinoa growing season 2017 are presented in Supplemental Table. S1.

2.2. Drought imposition and H_2O_2 treatments

Imposition of drought stress along with foliar application of H_2O_2 were initiated after four weeks of germination (from 15 July), when seedlings had completely established. Each genotype was divided into four groups viz. 1; Sufficient water supply without foliage applied H_2O_2 (Control, CK), 2; Sufficient water supply with foliage applied H_2O_2 (FAH), 3; Drought stress without foliage applied H_2O_2 (DS), 4; Drought

Table 1
Detail of four quinoa (*Chenopodium quinoa* Willd.) genotypes used in this study. **Source:** Main source was United States Department of Agriculture (USDA) since 2008.

Sr. No.	Plant Name	Code[a]	Origin
1	Colorado-407	PI596293	Colorado, USA
2	IESP	Ames 13730	New Mexico, USA
3	2-Want	Ames 13737	New Mexico, USA
4	Pichaman	PI634919	Chile

I collected from Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan.

[a] **Coding** as per the germplasm database (USDA).

stress with foliage applied H_2O_2 (DS + FAH). The control plants were irrigated at 75% of pot water holding capacity. Whereas drought stress was imposed at 30% of pot water holding capacity. Plants were irrigated on daily basis to maintain the treatment's water level. The H_2O_2 treatments were applied twice by foliage application at pre-optimized rate 15 mM (Iqbal et al., 2018). First application of H_2O_2 was carried out together with the imposition of drought stress (15 July) and the second was repeated after 15 days (30 July). Every time, non-treated plants were also sprayed with equal volume of distilled water as used for H_2O_2 application. Foliage application was carried out with compression sprayer (1 L capacity) to ensure even distribution of H_2O_2 solution on quinoa leaves. The experiment was terminated on 65th day after sowing (14 August). However, in the morning of termination day, fresh leaf samples were collected from intact plants and immediately stored at − 80 °C for further biochemical analysis.

2.3. Measurement of plant growth

Seedlings were gently uprooted and rinsed thoroughly with distilled water. Shoot length (SL) was measured by using measuring scale. After measuring fresh weight, shoot samples were oven dried at 70 °C for 72 h to measure their dry weights.

2.4. Measurement of chlorophyll and relative water contents (RWC)

For the determination of chlorophyll (a, b) and carotenoid contents, fresh leaves samples (0.2 g) were extracted in 80% acetone for overnight at 0–4 °C and then extract was centrifuged at 8000 × g for 10 min. Supernatant absorbance for carotenoids, chl *a* and chl *b* was observed at 480, 645 and 663 nm wavelength on spectrophotometer (Cary 60; Agilent, USA), respectively. For leaf relative water contents (RWC), leaf discs (5 mm in diameter) with 0.5 g fresh weight (FW) were put into distilled water for 4 h to get saturated weight (SW) and then dried in an oven at 85 °C for 24 h to get dry weight (DW). Thereafter, RWC was determined by using following formula.

$$RWC(\%) = (FW - DW) \div (SW - DW) \times 100$$

2.5. Measurement of antioxidant enzymes

2.5.1. Leaf extract preparation

The procedures for all enzymes extraction were carried out at 4 ^{0}C. Leaf samples (0.5 g) were crushed and ground with 2 mL extracting buffer (0.1 M potassium phosphate buffer with 0.1 mM EDTA, pH 7.0). Then extract was homogenized and centrifuged at 4 °C in a Beckman refrigerated centrifuge for 15 min at 15,000 × *g*. The supernatant was collected and stored at − 20 °C for enzyme activity measurements (Esfandiari et al., 2007).

2.5.2. Superoxide dismutase (SOD)

The procedure described by Giannopolitis and Ries (1977) was used to assess the activity of superoxide dismutase (SOD) by noting photo-reduction of nitroblue tetrazolium (NBT). Following it, 1.8 mL of 50 mM phosphate buffer (pH 7.8), 780 mL methionine (50 mM), 225 mL nitroblue tetrazolium (1 mM), 30 mL EDTA (10 mM), 150 mL riboflavin (1 mM) and 25 mL of protein extracts were added to plastic cuvettes. Then, cuvettes were exposed to light for 5 min and absorbance recorded at 560 nm using a spectrophotometer. The photo-reduction of NBT produces blue formazan. Superoxide dismutase (SOD) decreases photo-reduction, so the amount of enzyme used for 50% inhibition of NBT photo-reduction was considered as one unit of SOD activity.

2.5.3. Peroxidase (POD)

Peroxidase (POD) activity was determined on the basis of H_2O_2 peroxidation with guaiacol as an electron donor (Chance and Maehly, 1955). The reaction mixture contained phosphate buffer (50 mM, pH 7.0), hydrogen peroxide (40 mM), guaiacol solution (20 mM) and 100 µL crude enzyme. The increase in absorbance due to guaiacol oxidation was observed at 470 nm. The amount of enzyme causing a change in absorbance of 0.01 unit per minute was defined as one unit of POD activity.

2.5.4. Catalase (CAT)

For the measurement of catalase (CAT), 100 µL supernatant was mixed with 2.5 mL phosphate buffer (50 mM, pH 7.0) and 200 µL H_2O_2. The activity of CAT enzyme was accessed by H_2O_2 consumption, decrees in absorbance at 240 nm due to ingestion of H_2O_2 was observed after every 20 s (Chance and Maehly, 1955).

2.5.5. Glutathione peroxidase (GPX)

Glutathione peroxidase (GPX) activity was determined according to Paglia and Valentine (1967). The reaction mixture contained 0.1 M phosphate buffer (pH 6.8), 1 mM EDTA, 10 mM glutathione, 1 unit of glutathione reductase, 1 mM NaN_3 and 1.5 mM NADPH. After incubating for 10 min at 37 °C, H_2O_2 was added to each sample to a final concentration of 1 mM. The optical density was monitored at 340 nm and GPX activity was calculated as amount of NADPH oxidized.

2.5.6. Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity was assayed through catechol oxidation as suggested by Yu et al. (2014) with minor modifications. The reaction mixture contained 100 mM sodium phosphate buffer (pH 5.8), 50 mM catechol and 0.2 mL enzyme extract. The rate of catechol oxidation was monitored at 410 nm, and the amount of enzyme, causing an increase of 0.01unit per min was considered as one unit of PPO activity and expressed as unit min^{-1} g^{-1} FW.

2.5.7. Phenylalanine ammonia-lyase (PAL)

The phenylalanine ammonia-lyase (PAL) activity was measured according to Siriphanich and Kader (1985) with minor modifications, based on the formation of trans-cinnamic acid. The reaction mixture comprised of an enzyme extract (0.2 mL), 5 mM L-phenylalanine (1 mL) and 100 mM sodium phosphate buffer (pH 8.8). This mixture was spectrophotometrically measured in a quartz cuvette at the wavelength of 290 nm. The one unit of PAL was defined as the amount of enzyme needed to produce a change in absorbance of 0.01 per hour at 290 nm, which was equivalent to 1 mL reaction solution used to form 1 mg *trans*-cinnamic acid. The final activity of PPO was expressed as unit h^{-1} mg^{-1}fresh weight.

2.6. Measurement of proline content

Fresh leaf sample (0.25 g) was homogenized in 3% sulfosalicylic acid and centrifuged at 11,500 × *g*. Then, filtrate, acid ninhydrin, glacial acetic acid and phosphoric acid were taken together in test tubes and incubated at 100 °C for 1 h. Cooled in an ice bath, toluene was added and test tubes were shaken vigorously. After some time, chromophore containing toluene was read spectrophotometrically at 520 nm (Bates et al., 1973).

2.7. Measurement of total soluble sugar (TSS)

Total soluble sugar was appraised according to Razi et al. (1985). Leaves were ground in 80% ethanol, and centrifuged at 12,000 × *g*. Filtrate was mixed with 6 mL anthrone reagent and heated in boiling water bath for 10 min. Then, samples were ice-cooled, and incubated for 20 min at room temperature (25 °C). After incubation, optical density was recoded spectrophotometrically at 625 nm.

2.8. Ascorbic acid (AsA) contents

The AsA contents were measured following the method suggested

Table 2
Shoot length (cm), Shoot fresh weight (g), Shoot dry weight (g), Chlorophyll (*a* & *b*) and carotenoids contents (mg/g fresh weight) of quinoa genotypes influenced by hydrogen peroxide (H_2O_2) application under drought stress. Data were recorded at 65 DAS (days after sowing) and means were calculated from four replicates of each treatment; the values with different letters are significantly different by applying LSD test at $p \leq 0.05$.

	Treatments	SL	SFW	SDW	Chl a	Chl b	Crtd.
Pichaman	CK	60c	20.06c	7.05fg	0.73def	0.27defg	0.40fgh
	FAH	61c	20.31c	7.50efg	0.78de	0.31de	0.46def
	DS	35g	11.70e	4.00i	0.44h	0.13i	0.23k
	DS + FAH	40f	14.00de	4.53i	0.49gh	0.19ghi	0.27jk
IESP	CK	66b	23.89b	9.89cd	0.74def	0.29def	0.43efg
	FAH	66b	22.94b	10.11c	0.81de	0.35cd	0.50d
	DS	41f	15.60d	6.69g	0.53gh	0.18hi	0.38gh
	DS + FAH	47e	18.43c	7.62ef	0.60fg	0.21fghi	0.31ij
2-Want	CK	74a	33.93a	11.80b	1.35b	0.51b	0.68b
	FAH	77a	36.39a	14.50a	1.52a	0.65a	0.78a
	DS	51de	20.33c	7.90e	0.88cd	0.35cd	0.47de
	DS + FAH	55d	24.74b	9.30d	1.01c	0.40c	0.50d
Colorado-407D	CK	64bc	24.35b	9.90cd	0.97c	0.49b	0.59c
	FAH	61c	24.45b	9.61cd	0.99c	0.52b	0.57c
	DS	42f	13.79de	5.80h	0.63fg	0.18hi	0.35hi
	DS + FAH	48e	15.46d	5.91h	0.72ef	0.23efgh	0.42efg
LSD ($p \leq 0.05$)		4.3	2.57	0.77	0.15	0.08	0.06

CK, Sufficient water supply without foliage applied H_2O_2; **FAH**, Sufficient water supply with foliage applied H_2O_2; **DS,** Drought stress without foliage applied H_2O_2; **DS + FAH,** Drought stress with foliage applied H_2O_2.

by Mukherjee and Choudhuri (1983). Leaves samples (0.25 g) and 6% trichloroacetic acid (5 mL) were thoroughly mixed and filtrated. Then mixed with 1 mL of 2%, 2, 4-DNPH (2, 4 dinitrophenylhydrazine) followed by one drop of thiourea (10%). Samples were incubated at 100 °C in water bath for 15 min. After incubation, 2.5 mL of 80% H_2SO_4 added and optical density was noted at 530 nm.

2.9. Measurement of ROS production [H_2O_2 and superoxide radical ($O_2^{\bullet -}$)]

2.9.1. Hydrogen peroxide (H_2O_2)

Endogenous H_2O_2 concentration in leaves was measured by following the procedure described by Velikova et al. (2000). Leaf samples (0.25 g) were homogenized in 3 mL solution of TCA (5% trichloro acetic acid and 0.1 g charcoal) and then centrifuged at 12,000 × *g* for 15 min. To determine H_2O_2, supernatant (0.1 mL) was mixed with 1 M potassium iodide (1 mL) and 50 mM potassium phosphate buffer (1 mL). The absorbance of reaction mixture was then read spectrophotometrically at 390 nm.

2.9.2. Superoxide radical ($O_2^{\bullet -}$)

The method described by Elstner and Heupel (1976) with little modification was used to measure superoxide radicle ($O_2^{\bullet -}$) by monitoring the nitrate formation from hydroxylamine. Fresh leaf samples (0.3 g) were homogenized in 3 mL of 65 mM potassium phosphate buffer solution with pH 7.8, which was later centrifuged for 10 min at 5000 × *g and* 4 °C. The Supernatant was mixed with extraction buffer (65 mM phosphate buffer with pH 7.8) and 10 mM hydroxylamine hydrochloride, and then incubated at 25 °C for 20 min. After incubation, 17 mM sulfanilamide and 7 mM a-naphthylamine were added into mixture and again incubated at 25 °C for 20 min. Then, ethyl ether was added and vortex thoroughly. After some time, upper supernatant separated and optical density was read spectrophotometrically at 530 nm. The $O_2^{\bullet -}$ formation rate was calculated by $NaNO_2$ standard curves.

2.10. Determination of lipid peroxidation

The malondialdehyde (MDA) is a product of lipid peroxidation; its level was assessed through lipid peroxidation by following the method of Dhindsa et al. (1981) with minor modifications according to Yang et al. (2011). Fresh leaves samples (1.0 g) were homogenized in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 120,00 × *g* for 20 min., 2.0 mL of the supernatant was added with 2 mL of 0.67% thiobarbituric acid (TBA), and mixture was heated at 100 °C for 15 min and then cooled to room temperature. The absorbance due to aliquot was observed at 450 nm, 532 nm and 600 nm, then MDA contents were calculated by using following formula:

$$MDA = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

2.11. Measurement of inorganic ions

Oven dried leaf samples (1 g) were taken, dissolved into 100 mL miliQ water, heated for 30 min at 100 °C, cooled at room temperature and then obtained solutions were used to analyze the cations (Ca^{2+}, K^+, Mg^{2+} and Na^+) and anions (Cl^-, SO_4^{2-}) concentration. Inorganic ions concentration were measured through ion chromatography. The anions were determined by using plasma emission spectrometer (Agilent 735 ICP-OES, USA) and cations were measured on DIONEX ICS-5000 (USA).

2.12. Statistical analysis

The experiment was established in randomized complete block design (RCBD) with four replicates for each treatment. The replication data of each treatment were statistically analyzed using one-way analysis of variance (ANOVA) technique. Later, the significance between treatment means was tested with LSD (least significant differences) test at 5% probability level. All these statistical calculations were performed in a Windows statistical software *STATISTIX* (Version-8.1; Tallahasse, FL, USA). Furthermore, in order to obtain a multivariable view of all parameters and four genotypes under drought stress, as well as the interrelationships between all parameters, the relative mean values of all parameters for four genotypes were subjected to principal component analyses (PCA) using Origin Pro 9.1 software (OriginLab Corporation, Northampton, USA).

3. Results

3.1. Effect of drought and exogenous application of H_2O_2 on growth attributes of quinoa

Drought stress caused significant reduction in shoot length (SL), shoot fresh (SFW) and dry weight (SDW) in all quinoa genotypes

(Table 2). Inherently, 2-Want showed significantly higher plant height under both control and drought conditions. While, drought induced reduction in these parameters was the maximum in Pichaman followed by Colorado-407D. Nevertheless, 2-Want showed higher SL, SFW and SDW among all genotypes under both control and drought stress conditions. Foliage-applied H_2O_2 showed significant growth promoting effects particularly on SL and SDW production. Improvement in SL, SFW and SDW by H_2O_2 application was observed in all quinoa genotypes under drought conditions, while only 2-Want exhibited significantly higher SDW under control conditions (Table 2).

3.2. Effect of drought stress and exogenous application of H_2O_2 on chlorophyll (a & b) and carotenoid contents

Drought stress significantly reduced the chlorophyll a & b concentration in all quinoa genotypes (Table 2). Surprisingly, 2-Want genotype exhibited highest chlorophyll (a & b) and carotenoid contents under both control and drought stress conditions as compared with other genotypes. Exogenous application of H_2O_2 increased chl *a* (11.36%, 13.20%, 14.77%, 14.28%) and chl *b* (46.15%, 16.67%, 14.28%, 27.78%) under drought stress in Pichaman, IESP, 2-Want and Colorado-407D, respectively. Likewise, H_2O_2 also increased the carotenoid content under drought stress by 17.39%, 6.38% and 20% in Pichaman, 2-Want and Colorado-407D, respectively.

Nevertheless, H_2O_2 treated plants of IESP genotype showed reduced carotenoid concentration (18.42%) under drought stress.

3.3. Effect of drought stress and exogenous application of H_2O_2 on leaf relative water contents (LWC)

Drought and H_2O_2 treatments significantly affected the leaf relative water contents (LWC) of quinoa genotypes (Fig. 1C). It was observed that, 2-Want and IESP had higher potential to retain sufficient water in their leaves under both normal and drought conditions as compared to Colorado-407D and Pichaman genotypes. However, drought stress significantly decreased the LWC, while, application of H_2O_2 markedly improved the LWC by 22.74%, 12.61%, 7.34% and 14.28% in Pichaman, IESP, 2-Want and Colorado-407D, respectively (Fig. 1C).

3.4. Effect of drought stress and exogenous application of H_2O_2 on leaf total soluble sugar (TSS), proline and AsA contents

Total soluble sugars and proline contents increased significantly in all genotypes of quinoa in response to drought stress (Fig. 1A-B), no significant change was observed in total soluble sugars content of 2-Want genotype. Foliage-applied H_2O_2 further increased these accumulations (TSS, Pro.) and significant enhancement in TSS by H_2O_2 was found in all genotypes except Colorado-407D, while proline contents of Pichaman genotype remained unaffected by H_2O_2 application under drought stress.

Drought stress and foliage-applied H_2O_2 treatments resulted in a significant increase in AsA concentration of all quinoa genotypes (Fig. 1D). However, AsA contents in IESP and Colorado-407D genotypes did not influenced by DS and DS + FAH treatments, respectively. The highest AsA concentration was found in IESP (27.06 ± 1.64 mg/g FW) followed by Colorado-407D (22.6 ± 1.52 mg/g FW) under DS + FAH treatment.

3.5. Effect of drought stress and exogenous application of H_2O_2 on production of reactive oxygen species (H_2O_2 and $O_2^{\bullet -}$) and MDA contents

Drought stress significantly increased the ROS (H_2O_2 and $O_2^{\bullet -}$) concentration in leaves of all quinoa genotypes (Fig. 2A-B). Data revealed that, 2-Want genotype showed significantly lower

ROS concentration under drought stress as compared to other genotypes. The foliage-applied H_2O_2 significantly reduced H_2O_2 and $O_2^{\bullet -}$ concentration in all quinoa genotypes except in 2-Want, in which, reduction in endogenous H_2O_2 concentration was found statistically ($P \leq 0.05$) non-significant, relative to drought stress plants without H_2O_2 treatment. Pronounced production of ROS increased the MDA content under drought stress, while, foliar application of H_2O_2 significantly reduced the MDA content in all genotypes of quinoa. The lowest vale for MDA contents both under control and drought conditions was found in 2-Want followed by IESP genotype (Fig. 2C).

3.6. Effect of drought stress and exogenous application of H_2O_2 on inorganic ions Accumulation

Accumulation of inorganic ions (Cl^-, SO_4^{2-}, Ca^{2+}, K^+, Mg^{2+} and Na^+) in quinoa leaves of different genotypes was substantially changed under drought stress (Table 3). Drought significantly enhanced sodium ions (Na^+) concentration by 51.2%, 64.7%, 86.7% and 158.2% in Pichaman, IESP, 2-Want and Colorado-407D, respectively. Exogenous H_2O_2 application decreased these concentration by 10.4%, 5% and 32.8% in IESP, 2-Want and Colorado-407D, respectively. Contrastingly, 5.3% increased Na^+ concentration due to H_2O_2 application under drought stress was observed in Pichaman. Drought stress also increased chloride ions (Cl^-) concentration, which further improved by H_2O_2 application in all quinoa genotypes. Likewise, the calcium ions (Ca^{2+}) concentration also significantly increased due to drought stress and further enhanced by H_2O_2 application in all genotypes except Pichaman. However, contrary to drought, exogenous application of H_2O_2 decreased Ca^{2+} concentration in Pichaman by 57.3%. Moreover, in contrast with control, sulfate ions (SO_4^{2-}) concentration significantly improved in Pichaman (19.2%), IESP (13.9%), 2-Want (48.1%) and Colorado-407D (9.9%) under drought stress. However, this ions accumulation response of genotypes to exogenous H_2O_2 treatment under drought stress was contrasting. SO_4^{2-} concentration in Pichaman and 2-Want decreased by 5.2% and 16.9%, respectively, while in IESP and Colorado-407D, it increased by 2.5% and 31%, respectively. Drought stress improved magnesium ions (Mg^{2+}) concentration in Pichaman and Colorado-407D, while H_2O_2 application decreased Mg^{2+} concentration by 37.5% in Pichaman, 23.7% in Colorado-407D and 11.7% in IESP. However, decreased Mg^{2+} concentration under drought stress was significantly improved by H_2O_2 in 2-Want. Drought stress significantly enhanced potassium ions (K^+) concentration by 16%, 26.5%, 19.7% and 27.2% in Pichaman, IESP, 2-Want and Colorado-407D, respectively. Exogenous H_2O_2 application further increased these concentration by 11.8% in

Pichaman, 3.1% in IESP, 7.2% in 2-Want and decreased by 4.3% in Colorado-407D. Among all genotypes, the total accumulation of measured inorganic ions (Cl^-, SO_4^{2-}, Ca^{2+}, K^+, Mg^{2+} and Na^+), was found higher in 2-Want under both control and drought conditions. While, Pichaman showed lower potential to accumulate these ions under drought stress as compared to other genotypes. Moreover, drought stress markedly reduced K^+/Na^+ ratio in all quinoa genotypes. The Maximum reduction was found in Colorado-407D and the lowest in Pichaman. However, H_2O_2 application markedly improved the total concentration of measured ions and 2-Want exhibited pronounced accumulation of total ions with higher K^+/Na^+ ratio as compared to other genotypes under both normal and stressful conditions (Table 3).

3.7. Effect of drought stress and exogenous application of H_2O_2 on enzymatic antioxidant activity

Drought stress increased the SOD activity by 64.9%, 15.8%, 178.5% and 17.9% in Pichaman, IESP, 2-Want and Colorado-407D, respectively. However, increased SOD activity in IESP was found statistically ($P \leq 0.05$) non-significant with control (Fig. 3A). Application of H_2O_2 further enhanced the SOD activity in all genotypes and the highest SOD activity (25.11 ± 1.67 U mg Protein^{-1} FW) was found in 2-Want under DS + FAH treatment. The CAT activity increased in all genotypes

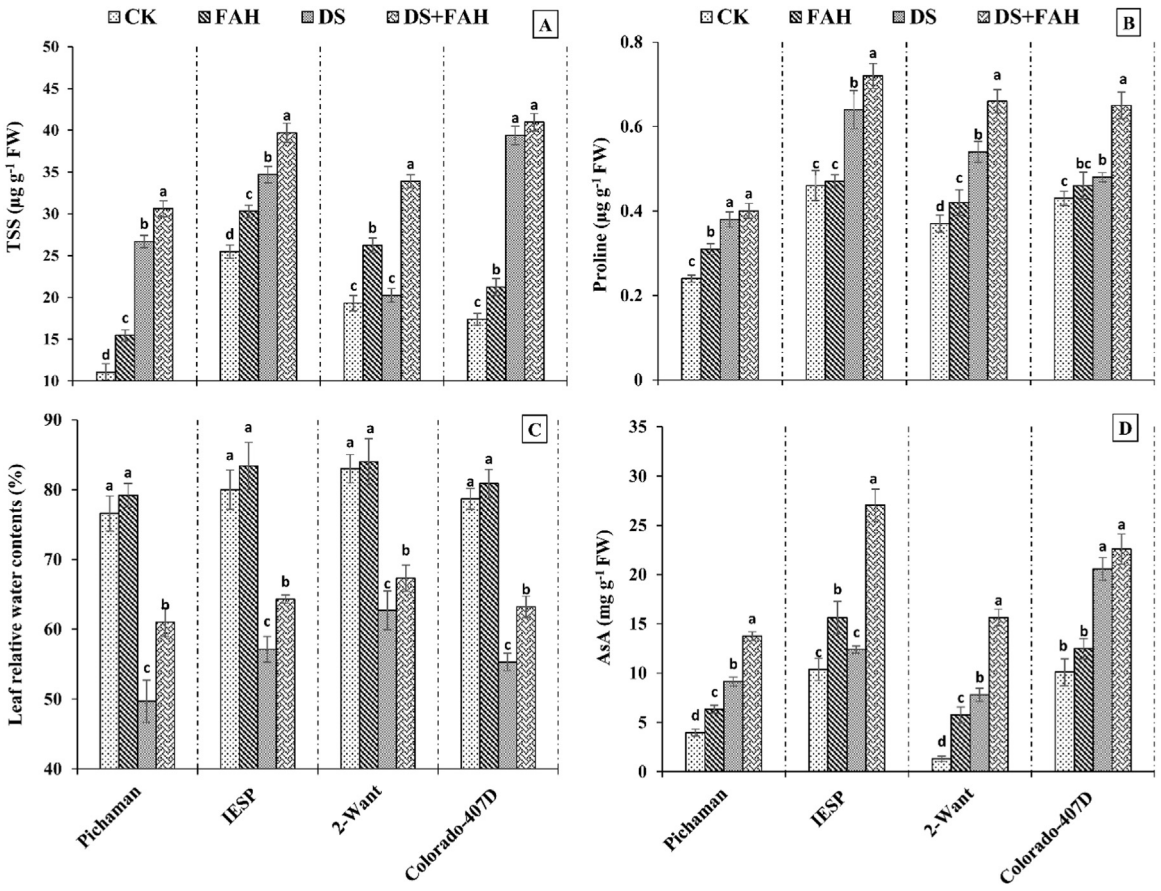


Fig. 1. Influence of foliage-applied hydrogen peroxide (H_2O_2) on concentrations of total soluble sugars (A), proline (B), leaf relative water content (C) and AsA (D) of quinoa genotypes under drought stress. Data were recorded at 65 DAS (days after sowing) from leaves tissues. All values are the means of four replicates and error bars represent SE values (n = 4). Different letters indicate significant differences ($P \leq 0.05$) among the treatments within each genotype. **CK,** Sufficient water supply without foliage applied H_2O_2; **FAH,** Sufficient water supply with foliage applied H_2O_2; **DS,** Drought stress without foliage applied H_2O_2; **DS + FAH,** Drought stress with foliage applied H_2O_2.

under drought stress, except Pichaman in which, CAT decreased significantly as compared to control. However, H_2O_2 application significantly enhanced the CAT activity under drought stress in all genotypes (Fig. 3C). The IESP and 2-Want genotypes showed increased POD activity, while Pichaman and Colorado-407D significantly decreased the POD activity under drought stress. However, exogenous application of H_2O_2 increased the POD activity by 91.3%, 43.3%, 7.8% and 29.3% in drought stressed Pichaman, IESP, 2-Want, and Colorado-407D quinoa genotypes, respectively (Fig. 3B). Drought and H_2O_2 treatment significantly improved the PAL and PPO activity in all genotypes as compared to control (Fig. 3D-E). However, the difference between DS and DS + FAH treatment was found statistically ($P \leq 0.05$) non-significant in 2-Want genotype for PAL activity. GPX activity markedly increased in 2-Want, IESP and Pichaman while, significantly decreased

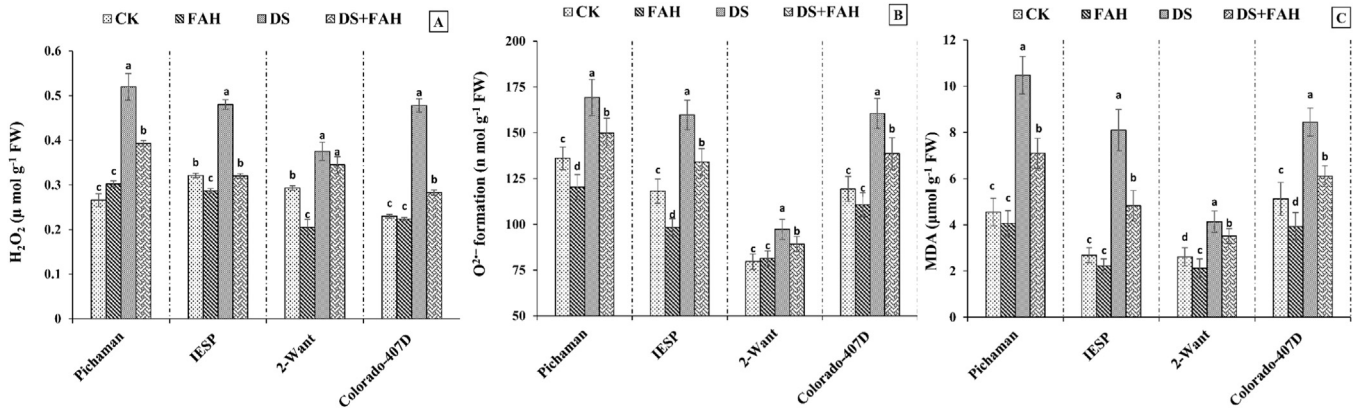


Fig. 2. Influence of foliage-applied hydrogen peroxide (H_2O_2) on endogenous H_2O_2 concentration (A) $O_2^{\bullet -}$ concentration (B) and MDA contents (C) of quinoa genotypes under drought stress. Data were recorded at 65 DAS (days after sowing) from leaves tissues. All values are the means of four replicates and error bars represent SE values (n = 4). Different letters indicate significant differences ($P \leq 0.05$) among the treatments within each genotype. **CK,** Sufficient water supply without foliage applied H_2O_2; **FAH**, Sufficient water supply with foliage applied H_2O_2; **DS,** Drought stress without foliage applied H_2O_2; **DS + FAH,** Drought stress with foliage applied H_2O_2.

Table 3
Accumulation of inorganic ions (Cl^-, SO_4^{2-}, Ca^{2+}, K^+, Mg^{2+} and Na^+) in quinoa genotypes as influenced by hydrogen peroxide (H_2O_2) application under drought stress. The concentrations of these inorganic ions are presented in mg/g on dry weight basis. Data were recorded at 65 DAS (days after sowing) and means were calculated from four replicates of each treatment; the values with different letters are significantly different by applying LSD test at $p \leq 0.05$.

	Treatments	Cl^{-1}	SO_4^{2-}	Ca^{2+}	K^+	Mg^{2+}	Na^+	Total Ions	K^+/Na^+
Pichaman	CK	09.63ef	07.60gh	0.18h	62.54gh	2.54f	1.25fg	83.75jk	49.89i
	FAH	09.78ef	07.57gh	0.28efg	60.46h	2.61ef	1.19g	81.89k	50.99i
	DS	17.93cd	09.06d	0.75b	72.57de	3.39ab	1.89b	105.59f	38.50j
	DS + FAH	18.07cd	08.59e	0.32e	81.16c	2.12g	1.99a	112.26e	40.86j
IESP	CK	08.95ef	09.75c	0.24fgh	64.38fgh	3.29bc	0.88hi	87.49ij	73.35de
	FAH	10.05ef	07.25i	0.22gh	68.81ef	3.12c	0.89h	90.34hi	77.60cd
	DS	17.24d	11.11b	0.31e	81.43c	3.24bc	1.45c	114.78de	55.97hi
	DS + FAH	21.89b	11.39a	0.39d	83.95bc	2.86d	1.30ef	121.76bc	64.52fg
2-Want	CK	10.08e	07.36hi	0.20h	72.16de	3.56a	0.75j	94.11gh	96.44a
	FAH	09.85ef	07.75g	0.34de	76.07d	2.24g	0.79j	97.38g	95.90a
	DS	23.17b	10.90b	0.54c	86.36b	2.79de	1.40cd	125.16b	61.78gh
	DS + FAH	27.93a	09.06d	0.75b	92.57a	3.39ab	1.33def	135.07a	69.78ef
Colorado-407D	CK	08.51f	08.05f	0.30ef	66.81fg	2.12g	0.79j	86.59ijk	84.93b
	FAH	09.63ef	07.60gh	0.18h	62.54gh	2.54f	0.75j	83.22jk	83.0bc
	DS	17.50d	08.85de	0.56c	84.96bc	3.29ab	2.04a	117.2cd	41.61j
	DS + FAH	19.13c	11.59a	1.71a	81.29c	2.51f	1.37cde	117.60cd	59.01gh
LSD ($p \leq 0.05$)		01.55	00.26	0.07	04.46	0.18	0.09	4.83	07.32

CK, Sufficient water supply without foliage applied H_2O_2; **FAH**, Sufficient water supply with foliage applied H_2O_2; **DS,** Drought stress without foliage applied H_2O_2; **DS + FAH,** Drought stress with foliage applied H_2O_2.

in Colorado-407D genotype under drought stress (Fig. 3F). However, application of H_2O_2 significantly improved the GPX activity by 54.7% in drought stressed plants of Colorado-407D.

3.8. Co-relations

The K^+/Na^+ ratio in quinoa genotypes were positively correlated with shoot fresh ($R^2 = 0.836$) and shoot dry weight ($R^2 = 0.842$) in the present study (Fig. 4A). The PC 1 and PC 2 of principle component analysis (PCA) explained 42.48% and 31.68% of the total variability, respectively (Fig. 4B). Association between osmolytes, oxidative damages, antioxidants and growth traits were explored with the angles between traits vectors. The angle cosine among two traits vectors indicates correlation between traits, obtuse and acute angles indicate negative and positive association, respectively. The growth parameters (SL, SFW, SDW, Chl *a*, Chl *b*, and carotenoids), osmolytes (K^+, Cl^-, Na^+/K^+, Proline) and antioxidants (SOD, POD, PAL and CAT) were grouped together with acute angle in 2-Want and IESP genotypes. These parameters positively contributed for both genotypes in drought tolerance, 2-Want was highly favored by SOD, POD, PPO and AsA, while, IESP genotype mediated by CAT and PAL. Reactive oxygen species (H_2O_2 and $O_2^{\bullet -}$) and toxic ions concentration (Na^+ and Mg^{2+}) were positively correlated with MDA and grouped together in Colorado-407D genotype. The vectors of GPX and PPO originated towards Pichaman with acute angle, while these vectors shows obtuse angles with growth traits.

4. Discussion

Quinoa is a drought resilient crop, but its performance reduces under water-deficit conditions (Iqbal et al., 2018). The magnitude of adverse impact of drought on quinoa performance mainly depends on variety (genotype), degree of stress and also influenced by other environmental conditions (Sun et al., 2014; Iqbal et al., 2018). Drought induced reduction in SL, SFW, and SDW was more prominent in genotypes Pichaman and Colorado-407D than 2-Want and IESP. This growth inhibition in these genotypes could be attributed to higher oxidative damages (Fig. 2C), which corresponds with Aziz et al. (2018) and Iqbal et al. (2018), whom also reported for drought stressed quinoa. Similarly, Iqbal et al. (2017) also observed more drastic reduction in shoot biomass production in Pichaman than 2-Want under salt stress. On the other hand, several studies have reported that drought exerts oxidative stress on plants by excessive formation of ROS like H_2O_2 and $O_2^{\bullet -}$, which can potentially damage the cellular membranes, causing lipid peroxidation that is evident from the present study (Fig. 2) as well, and leads to inhibition of growth performance in many plant species (Anjum et al., 2015; Cruz de Carvalho, 2008). Malondialdehyde (MDA) contents are often used to measure the level of lipid peroxidation resulting from oxidative stress (Smirnoff, 1993). However, the excessive formation of ROS beyond threshold level increases lipid peroxidation (MDA) which has been observed in numerous crops such as wheat, barley and quinoa under drought stress (Ahmed et al., 2013; Amjad et al., 2015; Aziz et al., 2018; Farooq et al., 2017). These findings accord with the results of present study, where increased H_2O_2 and $O_2^{\bullet -}$ induced higher level of MDA under drought stress in all quinoa genotypes (Fig. 2). Although, 2-Want exhibited lower production of ROS and MDA than the other genotypes suggesting that 2-Want has higher tolerance to drought stress than others.

Reduction in pigments concentration has been considered as an indicator of oxidative stress (Smirnoff, 1993). Under drought stress, the highest and lowest reduction in pigment contents (Chl a, Chl b and carotenoids) were observed in Pichaman and 2-Want genotypes, respectively (Table 2). These results indicate that drought induced oxidative damages were more pronounced in Pichaman than other genotypes. However, foliage applied H_2O_2 significantly reduced the oxidative damage (Fig. 2C) by decreasing H_2O_2, $O_2^{\bullet -}$ concentration, and increasing pigment contents which lead to an increased plant growth (SL, SFW, SDW) under drought stress in all quinoa genotypes. Hossain and Fujita (2013) reported that exogenous applied H_2O_2 induces both ROS and methylglyoxal (MG) detoxification pathways, which reduce the MDA and endogenous H_2O_2 levels in mustard under water deficit conditions. Studies have also shown that exogenous H_2O_2 application can enhance drought tolerance in several crop plants such as wheat, maize, soybean and quinoa by increasing osmolytes accumulation and improving antioxidant activity (Farooq et al., 2017; Iqbal et al., 2018; Ashraf et al., 2015; Guler and Pehlivan, 2016). In the present study, reduced oxidative damages due to H_2O_2 application, which led to an improved plant growth could be associated with better osmotic adjustment and improved antioxidant activity under drought stress (Table 2, Fig. 3).

A wide range of osmolytes including inorganic (Na^+, K^+, Cl^-) and organic ions (proline, soluble sugar, glycine betaine) contribute in osmotic adjustment (OA) of plants (Blum, 2017). The increased levels of proline and soluble sugar can mitigate water loss, regulate turgor

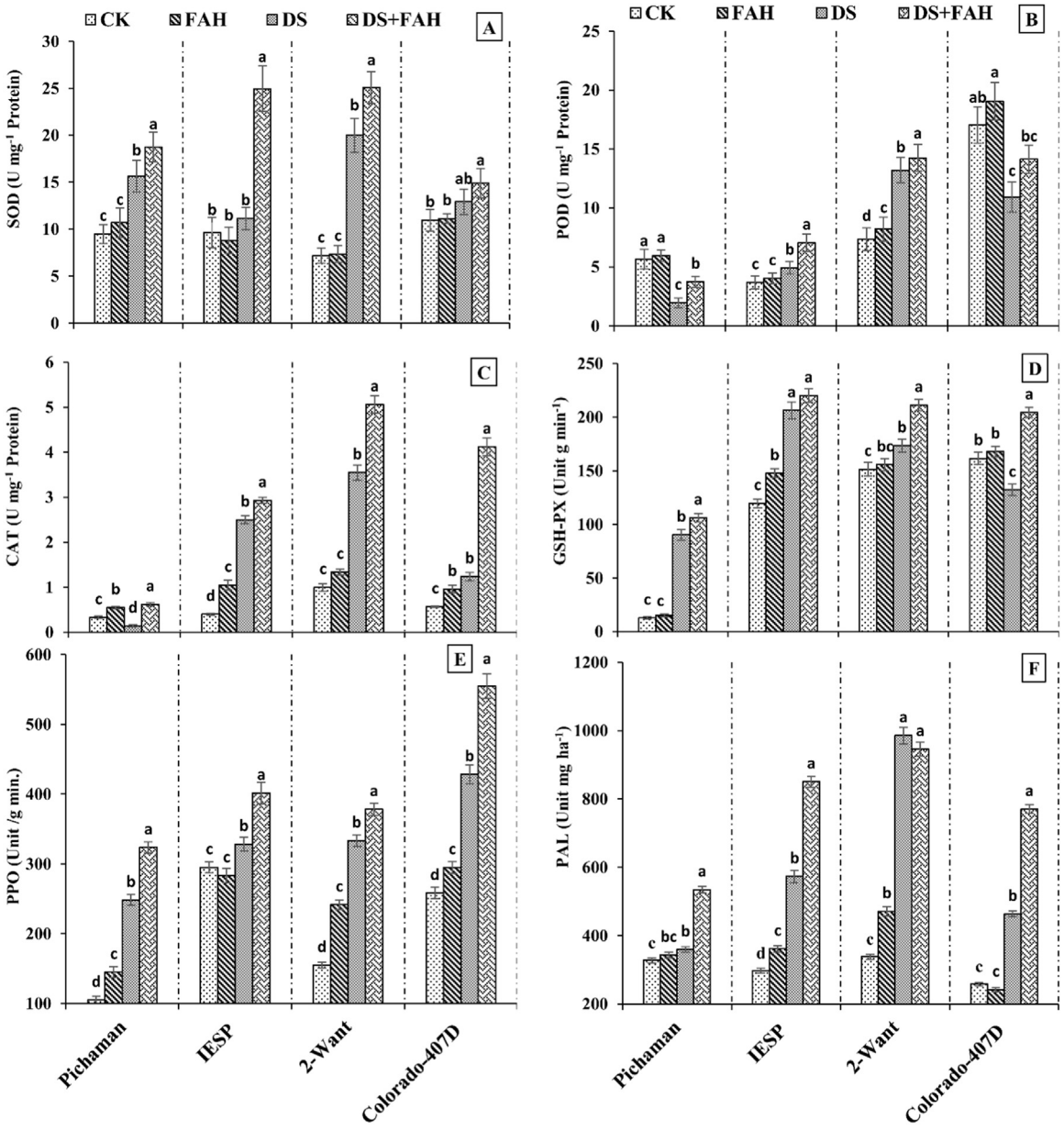
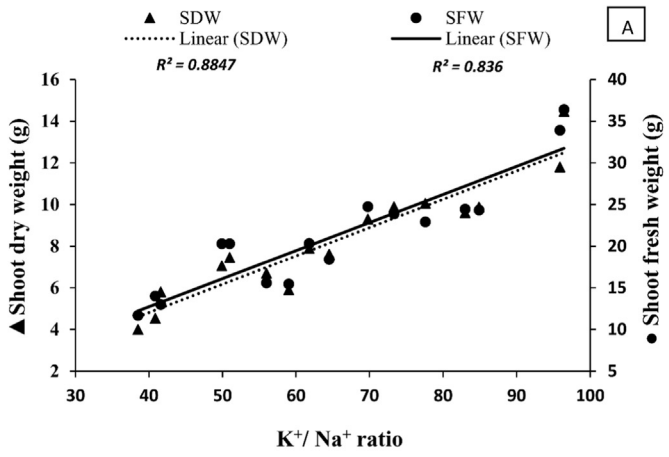


Fig. 3. Influence of foliage-applied hydrogen peroxide (H_2O_2) on SOD (A), POD (B), CAT (C), GSH-PX (D), PPO (E) and PAL (F) activity of quinoa genotypes under drought stress. Data were recorded at 65 DAS (days after sowing) from leaves tissues. All values are the means of four replicates and error bars represent SE values (n = 4). Different letters indicate significant differences ($P \leq 0.05$) among the treatments within each genotype. **CK**, Sufficient water supply without foliage applied H_2O_2; **FAH,** Sufficient water supply with foliage applied H_2O_2; **DS,** Drought stress without foliage applied H_2O_2; **DS + FAH,** Drought stress with foliage applied H_2O_2 .

potential, and maintain cell membrane integrity to improve growth under water deficit conditions (Gallie, 2012; Rodziewicz et al., 2014). Pronounced synthesis of proline and TSS under drought stress as evident in the present study (Fig. 1A-B) accords with previous reports on quinoa (Aziz et al., 2018). Application of H_2O_2 further improved the proline and TSS contents under drought stress in quinoa genotypes that corresponds with Terzi et al. (2014) in maize and Farooq et al. (2017) in bread wheat. Moreover, according to Terzi et al. (2014), the pre-treatment with H_2O_2 improves osmotic stress tolerance in maize by increasing the level of osmolytes (proline, polyamines and TSS), thereby increasing leaf water status. Likewise, significantly higher relative leaf water contents responded by quinoa genotypes by H_2O_2 application (Fig. 1C), corresponds with Ishibashi et al. (2011) and Iqbal et al. (2018), which could be plausible with increased accumulation of osmolytes under drought stress (Fig. 1).

The higher rate of proline and TSS sugar accumulation seemed to be associated with drought tolerance in several plants (Liu et al., 2011), and significantly higher rate of these accumulation was found in IESP genotype of the present study. In contrast, drought stress did not affect TSS content in 2-Want, while the highest LWC was found in 2-Want genotype (Fig. 1C). However, this higher leaf water status in 2-Want might be associated with osmotic adjustment, mainly through increased inorganic ions accumulation (Table 3) rather than organic ions under drought stress.

The accumulation of inorganic ions and their contribution in OA was not previously explored under drought stress in quinoa. Orsini et al. (2011) reported that Na^+, K^+ and Cl^- were the three most represented inorganic ions for OA in quinoa. In this study, accumulation of K^+ and Cl^- was observed markedly higher in 2-Want than those of other genotypes (Table 3). Similarly, Sun et al. (2014) reported that, re-watering after progressive drought increased the accumulation of inorganic ions in quinoa genotypes and found higher biomass in Titicaca who uptakes



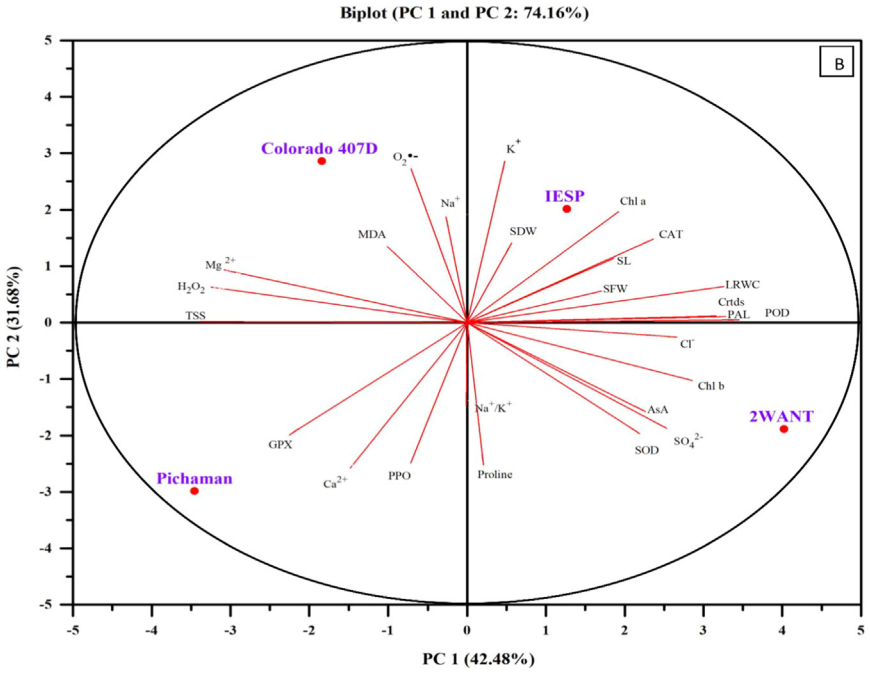


Fig. 4. (A) Relationship of K^+/Na^+ ratio with shoot fresh and shoot dry weight of quinoa genotypes. Each circle/triangle in figure represent the treatment means value of different genotypes. The values are means of four replicates. **(B)** A principle component analysis (PCA) showing relative contribution of all physio-biochemical attributes in four quinoa genotypes under drought stress. The first two components (PC 1 and PC 2) explained 42.48% and 31.68% of total variations between the studied variables respectively. Correlation among traits were interpreted through the angles between variables vector lines. The acute and obtuse angles between the trait vectors indicate positive and negative correlations respectively. Narrow the acute angle or wider the obtuse angle; greater the correlation coefficient, while, right angle between the vectors of two variable indicates no correlation between them.

more ions. Therefore it may be inferred that, higher accumulation of inorganic ions in 2-Want and pronounced synthesis of proline and TSS in IESP improved osmotic adjustment, leading to leaf expansion (Liu et al., 2003; Volkenburgh, 1999), which ultimately resulted in higher biomass accumulation SFW and SDW as compared to Pichaman and Colorado-407D.

The K^+/Na^+ ratio is considered as an index of salinity tolerance, and has been well documented in numerous plants like quinoa (Hariadi et al., 2010; Shabala et al., 2012), while, the association of this ratio in drought tolerance is still unclear. In current study, 2-Want exhibited significantly higher K^+/Na^+ ratio than Colorado-407D and IESP under both control and drought conditions, respectively. However, positive interaction between K^+/Na^+ ratio and dry mass production in the present study showed the significance of K^+/Na^+ ratio (Fig. 4A) in drought tolerance of quinoa.

The antioxidant system is closely related to scavenge cytotoxic ROS concentration when the redox balance is disturbed under stressful conditions. Among antioxidants, superoxide dismutase (SOD) is known as first line of defense against oxidative stress and its activity has been reported to increases in several plants exposed to environmental stresses, including drought and salinity (Ahmed et al., 2013; Amjad et al., 2015; Fghire et al., 2013). SOD plays a key role against superoxide ($O_2^{\bullet -}$) by dismutaseing it to H_2O_2 and O_2. Previously, a correlation between stress tolerance and SOD activity has been well documented (Hamed et al., 2007), and enhanced oxidative stress tolerance as results of increased SOD production has also been reported in quinoa (Amjad et al., 2015). However, concerning this study, drought stress increased SOD production in quinoa genotype, while 2-Want exhibited significantly higher SOD activity than other genotypes, revealing the highest defense potential of 2-Want against the oxidative stress under drought.

The antioxidant system works in a coordinated manner, as superoxide neutralize by SOD, while, at the same time H_2O_2 is produced as result of SOD activity, which is itself cytotoxic (Foyer and Noctor, 2000). The over generation of H_2O_2 is neutralize to H_2O by a group of antioxidant enzymes including CAT, POD and GPX (Jaleel et al., 2007). In this study, the concentration of all of these enzymes (CAT, POD and GPX) significantly increased under drought stress, more specifically in 2-Want and IESP genotypes that corresponds with Fghire et al. (2013), Amjad et al. (2015) and Aziz et al. (2018), whom reported in quinoa. While, contrary to that, Pichaman genotype exhibited significantly decreased CAT and POD, and Colorado-407D showed decreased POD and GPX activities under drought stress, indicating that successful coordination of additional anti-oxidative enzymes was impaired in these

genotypes as compared to 2-Want and IESP. Previously, Waqas et al. (2017) and Iqbal et al. (2018) also observed decreased CAT and POD activity in Pichaman genotype under salt and drought stress, respectively.

Secondary metabolites such as phenols and flavonoids play an imperative role in ROS scavenging and alleviate oxidative stress in plants (Wang et al., 2011). The antioxidant enzymes related secondary metabolic activities such as PAL and PPO were significantly increased and further improved by H_2O_2 application in all genotypes under drought stress in the present study (Fig. 3E-F). It has been found that, increased PAL and PPO activities stimulate the phenylpropanoid pathways (Farooq et al., 2016) and produce phenylpropanoid derivatives such as phenols and flavonoids (Ahmed et al., 2013), which results in reduced oxidative damage under drought and salinity stress (Ahmed et al., 2015). In addition, some non-enzymatic antioxidant (ASA and GSH) also play a vital role in ROS detoxification. Ahmed et al. (2013) documented that ROS scavenging was improved through enhanced AsA concentration in barley genotypes under drought and salinity stress. Similarly, a significant increase in ASA concentration was observed in drought stressed plants of all quinoa genotypes in the present study, except IESP.

However, the decreased CAT, POD and GPX activity in Pichaman and Colorado-407D were significantly enhanced by exogenous H_2O_2 application under drought stress. Additionally, H_2O_2 application further improved the enzymatic and non-enzymatic antioxidants in all quinoa genotypes. In the line of that, several former elucidations have also reported an enhanced H_2O_2 induced antioxidants activity in maize (Ashraf et al., 2015), soybean (Guler and Pehlivan, 2016), Cucumber (Liu et al., 2010) and mustard (Hossain and Fujita, 2013).

Hydrogen peroxide (H_2O_2) as a stress signaling molecule (Farooq et al., 2017; Hossain et al., 2015; Bienert et al., 2006; Shu-Hsien et al., 2005) is not only involved in the regulation of gene expression (Hossain et al., 2015; Abass and Mohamed, 2011; Hossain and Fujita, 2013; Shu-Hsien et al., 2005; Ishibashi et al., 2011),but also associated with plant defense in terms of antioxidants, defense and signaling proteins (Wahid et al., 2007) and activates the ROS and methylglyoxal (MG) detoxification pathways as well (Hossain and Fujita, 2013). Therefore, it could be inferred that exogenously applied H_2O_2 evokes the signaling pathways and increases the antioxidant activities, leading to decreased oxidative damage, which is evident in this study (Fig. 2).

The results clearly indicate that 2-Want and IESP genotypes exhibited higher antioxidant activity as compared to Colorado-407D and Pichaman under drought stress. The biplot of PCA revealed many important biochemical parameters as powerful explanatory variables, which were responsible for drought tolerance in quinoa, suggesting that they could be useful for genotypes screening at early developmental stages. In particular, better accumulation of osmolytes (Proline, K^+, Na^+/K^+, Cl^-) and photosynthetic pigments (Chl *a*, Chl *b*, Cartds) with improved antioxidants (CAT, SOD, POD, AsA) are the promising traits, which could be used as potential selection markers for screening out the drought tolerant genotypes of quinoa. Therefore, it could be conceivable and reasonable to suggest that drought tolerance potential of 2-Want and IESP genotypes was much higher than Pichaman genotype which showed sensitivity to drought stress. The Colorado-407D genotype exhibited higher variations as compared to other genotypes, thus, found as a moderately drought tolerant genotype.

5. Conclusion

Overall, drought stress (30% WHC) reduced the quinoa performance in all genotypes, while, 2-Want and IESP performed better and exhibited higher plant growth and biomass production as compared to Colorado-407D and Pichman genotypes. Drought tolerance was linked with better osmotic adjustment and pronounced antioxidant activity. Nevertheless, foliage-applied H_2O_2 (15 mM) significantly improves the osmotic adjustment through better osmolytes accumulation, enhances antioxidant activity and improves K^+/Na^+ ratio, which increase water relations, reduce lipid peroxidation and ultimately result in better growth and development of quinoa genotypes under drought stress. Thus, it is suggested that foliage application of H_2O_2 may be opted to improve osmotic adjustment and antioxidant system of quinoa in order to enhance its drought tolerance, even in sensitive genotypes, such as Pichaman. In addition, this study also suggests that the screened out drought-resilient genotypes could also be widely adopted on the marginal lands of water scarce regions.

Acknowledgement

This study was financially supported by the Chinese Academy of Sciences (CAS)–the Academy of Sciences for the Developing World (TWAS) President's Fellowship Program for international Ph.D. students (awardee of 2015 CAS-TWAS President's Fellowship) and the Strategic Priority Research Program of the Chinese Academy of Sciences, China, Grant no. XDA19030204.

Conflict of interest

The authors declare that they have no conflict of interest

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.08.004.

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